The Neuromuscular Response to Eccentric Exercise-Induced Muscle Damage in Younger and Older Females: A Preliminary Study

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Abstract

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The aging process is associated with many functional decrements. It is important to consider the additive acute functional decreases that may occur following eccentric exercise-induced muscle damage (EIMD). This study aimed to examine the effect of age on muscle damage metrics following EIMD. Ten younger (age=20±2 years, BMI=22.49±3.97 kg/m2) and six older (age=68±2 years, BMI=24.45±2.82 kg/m2) females participated in this study. Participants performed a maximal knee extensor EIMD protocol. Additionally, participants completed various assessments before, immediately after, 24-, 48-, and 72-hours after the EIMD protocol to evaluate the presence of and recovery from muscle damage. Assessments included B-mode ultrasound imaging of the rectus femoris (RF) and vastus lateralis (VL) to assess cross sectional area (CSA) and echo intensity (EI), soreness measures, and neuromuscular assessments (i.e., peak torque [PT] and rapid torque [RT]). A 2x5 ANOVA was used to compare age groups by time for all variables. Significant age x time interactions were found for RT at 200ms and 250ms ($p<0.001$) indicating a slower rate of recovery for the older group. Significant main effects for age were found for PT ($p<0.001$), maximal eccentric torque ($p<0.001$), VL CSA ($p<0.001$), RF CSA ($p=0.03$), RF EI ($p=0.03$), and all soreness measures ($p<0.05$). Overall, there were significant differences in performance, muscle morphology, and soreness between the younger and older females as well as significant differences in the late-phase RT recovery timeline. Practitioners should remain aware of the decreased functional abilities of female clients as they age and prescribe exercises appropriately.

Keywords: Aging, dynamometry, ultrasound, EMID

Introduction

Older adults are a prominent and growing age group within the United States. As of 2018, there were 52 million people over the age of 65 living in the United States which accounts for 16% of the total population.[1] It is projected that by the year 2030, there will be 73 million older adults representing 21% of the total population.[1] These older adults are at an increased risk of injury. Indeed, in 2018, 27.5% of older adults reported falling at least one time in the previous year; of which, 10.2% sustained a fall-related injury.[2] Sustaining a fall-related injury results in a

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decreased quality of life and reduces the ability to perform activities of daily living, eventually leading to a loss of independence.

A primary physiological factor which contributes to falls in older adults is dynapenia, the age-related loss in strength and power.[3] Also associated with the aging process is sarcopenia, the age associated decrease in muscle mass which can contribute to the decrease in muscle strength.[3,4] Females in specific tend to lose ~3.5% of their isometric leg extensor strength per year during the 6th to 8th decade of life.[5] Throughout aging, spinal motor neuron apoptosis occurs, resulting in muscle fiber denervation from the alpha motor neurons[6] The loss of motor units and changes to existing motor unit function may also contribute to the loss in strength seen throughout the lifespan.[6,7] Denervation preferentially affects type II muscle fibers, resulting in atrophy and decreased strength.[6,7]

Muscle strengthening activities are often encouraged for aging populations in order to minimize the progression of dynapenia. From the early 2000's to 2022, the number of older adults who met the suggested guidelines for muscle strengthening activities increased from 11.7% to 22%.[8] However, with the growing percentage of active older adults, it is important to consider the additional neuromuscular performance decrements, such as decreased force generating capacity, that may occur following intense unaccustomed exercise. Since muscle strength and power are associated with the ability to remain independent, a decrease in force generating capacity throughout the recovery period following muscle damage may hinder the ability for older individuals to perform activities of daily living.[9] Reductions in muscle strength may also result in an increased fall risk, which is greater in females than in males.[9] Due to the role of strength in the ability to perform activities of daily living and fall risk, it is important to explore how age affects the degree to which decreases in functional performance occur and the recovery timeline following intense unaccustomed exercise, especially in females.

Muscle damage may be a result of performing unaccustomed exercise or work-related activities that include eccentric muscle actions (i.e., lifting and carrying heavy boxes or walking up and down several flights of stairs, etc.). The forceful lengthening of the muscle during an eccentric muscle action results in excess mechanical tension of sarcomeres.[10] The sarcomere strain results in Z-line streaming and myofibrillar disruption which may be exacerbated during the initial stages of recovery. There are several methods to quantify the damage produced from a series of eccentric muscle actions. Methods of assessing muscle damage include, but are not limited to, muscle strength[11] and rate of torque development[12] to quantify functional decrements, cross-sectional area using Brightness mode (B-mode) ultrasonography assessments[13] to assess muscle swelling, and muscle soreness[14] including palpation.[15] All of the aforementioned measures provide the ability to track initial muscle damage as well as progression and recovery following a muscle damage inducing protocol.

Age related changes in recovery from eccentric muscle damage are not well understood. At the cellular level, muscle regenerative capacity decreases with age. However, this may not always translate to substantial differences in younger versus older adults. For example, previous research has shown no differences in the loss or recovery of peak torque after eccentric elbow flexor muscle damage in older versus younger males.[16] Other work has reported that older males experience greater lower-body muscle damage than younger males.[17] However, the authors reported no difference in the pattern of recovery between older and younger males.[17] In a study by Dedrick & Clarkson,[18] younger and older females performed eccentric elbow flexor exercises. Their results showed that the younger females' strength recovered quicker than the older females.[18]

While limited research has investigated age related differences in recovery from eccentric muscle damage of the upper body, research has not yet explored the effect of ages on recovery from eccentric muscle damage of the lower body. Evaluating the neuromuscular recovery from lower body EIMD is also important due to the possible increased fall risk as a result of muscle damage. Further research is needed to explore these effects in young and older adults, and in particular, females. Therefore, the primary aim of this study was to evaluate the effect of age on the decline in, and recovery of, isometric peak torque, eccentric peak torque, and rapid torque following EIMD. It was hypothesized that the older adults would experience greater declines in peak and rapid torque and the younger females will recover to initial peak and rapid torque values sooner than the older females. A secondary aim of this study was to quantify the effect of age on muscle size and quality following eccentric muscle damage. It was hypothesized that the older and younger females would experience similar changes in muscle size and quality following EIMD. In addition, it was hypothesized that both groups would demonstrate similar recovery patterns. These hypotheses were supported by Nosaka & Clarkson[19] which reported an increase in upper body muscle thickness following EIMD. Finally, the tertiary aim of this study was to determine the effect of age on subjective (soreness) metrics of functional performance following eccentric muscle damage. It was hypothesized that the older females would report less soreness and return to initial subjective values sooner.

Methods

Participants

Ten younger (age = 20 ± 2 years, body mass index (BMI) = 22.49 ± 3.97 kg/m^2) and six older (age = 68 ± 2 years, BMI = 24.45 ± 2.82 kg/m^2) females enrolled in this study. The demographics and anthropometrics for the groups are presented in Table 1. In both groups, one female withdrew after the 48-hour follow up, resulting in nine younger females and five older females to complete the full study. A diagram showing the path of recruitment can be seen in Figure 1. In order to account for menstrual cycle, all young females (ages 18-35) were required to have regular menstruation.[20] Regular menstruation was defined as either a natural regular menstrual cycle or the use of oral, hormonal contraceptive pills throughout the full duration of the study.[20] Testing took place within the first two weeks of the onset of menstruation. The older adults (ages 65-80) were required to not currently be on any hormone replacement therapies throughout the study. Participants were excluded from the study if they had a body mass index ≥ 30 kg/m^2, had a history of lower extremity surgery, required the use of an assistive walking device, experienced a myocardial infarction within the year prior to participation, or had any known neuromuscular or metabolic disease(s).[21] Physical activity limitations were as follows: six months prior to participation, participants must have performed resistance training less than three times per month and refrained from other structured exercise consisting of more than 30 min/d, three times per week. This study was approved by the university Institutional Review Board for human subject research. This research was carried out fully in accordance with the ethical standards of the International Journal of Exercise Science.[22]

Sample size was determined using an *a priori* power analysis based on changes in peak torque following eccentric muscle action induced muscle damage in previously published studies. Effect sizes were calculated from the mean and standard error of maximal isometric strength from Dedrick & Clarkson [16] (Cohen's d = 0.196) and Jamurtas et al. [23] (Cohen's d = 0.12). Additionally, Levitt et al. [20] reported partial eta squared of 0.18 for the main effect of time in maximal quadriceps isometric strength in younger females. Collectively, based on these previous investigations, we

used the following parameters to power the present study: ANOVA (within-between interaction effect) analysis, a conservative effect size estimate of f = 0.468, power of 0.8, two groups, five measurements, a correlation among repeated measures of 0.5, and a nonsphericity correction of 1.0. The results from the G*Power software (Version 3.1.9.7) *a priori* effect size estimation procedures indicated that a minimum of eight total participants (four in each group) were needed. However, in order to account for participant dropout and conservatively adjusting the effect size for additional power, the aim was to recruit 10 participants per group.

Table 1. Participant Demographics and Anthropometrics. This table shows the demographic and anthropometric data for participants by age group (Mean ± SD).

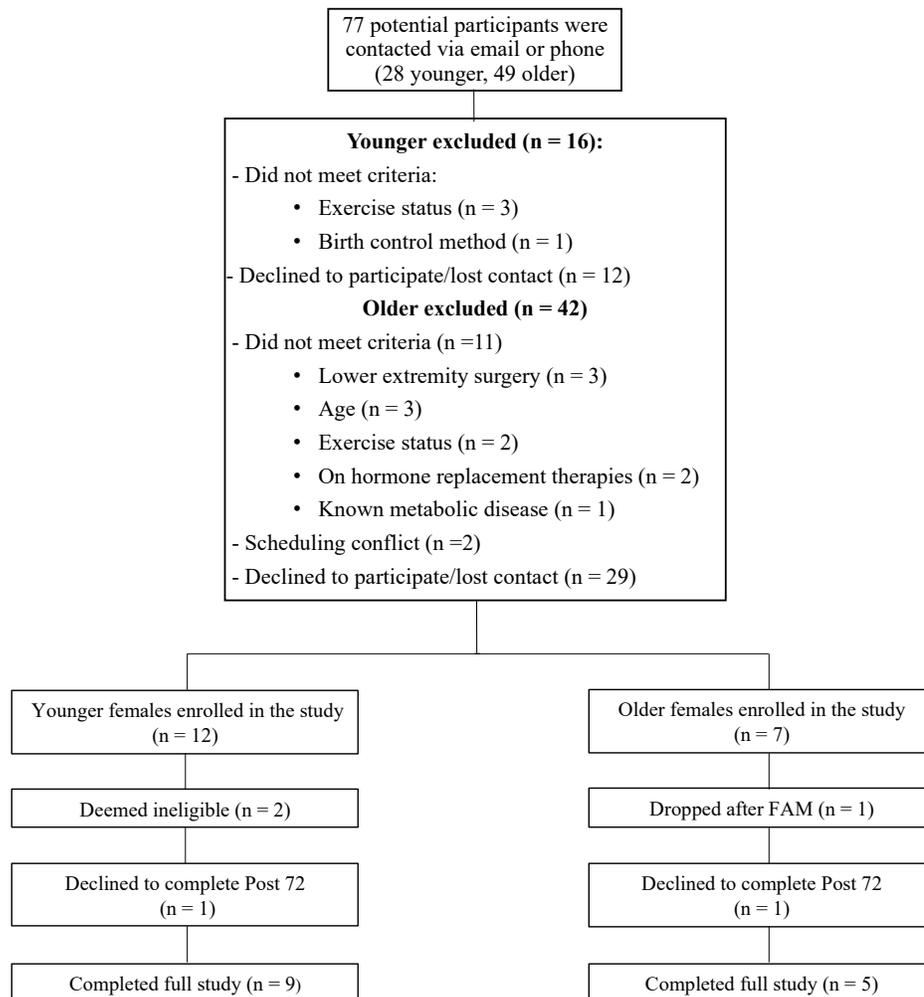| *Demographics*	**Younger (n = 10)**	**Older (n = 6)**
Age	20 ± 2	68 ± 2
% (n) White	80.00 (8)	83.33 (5)
% (n) Hispanic	10.00 (1)	16.67 (1)
% (n) Asian	10.00 (1)	0.00
Anthropometrics		
Height (cm)	168.63 ± 4.80	163.02 ± 6.19
Mass (kg)	63.80 ± 12.07	64.73 ± 5.74
BMI (kg/m²)	22.49 ± 3.98	24.45 ± 2.82

Figure 1. Diagram for Study Recruitment and Enrollment. A flow chart demonstrating recruitment status amongst the older and younger females.

Protocol

The study consisted of five visits to the laboratory (Figure 2). Visit one served as a familiarization day in which participants completed paperwork consisting of an informed consent document and a health history questionnaire. Following the paperwork, participants were familiarized with the neuromuscular assessments including maximal voluntary contractions (MVC) and rapid isometric muscle actions (rapid torque [RT]). Participants were also familiarized with maximal isokinetic eccentric muscle actions (ECC) for the eccentric strength assessment, but only three trials were performed in an effort to prevent muscle damage during familiarization. All neuromuscular assessments were performed on the participant's dominant leg determined by asking the participant which leg they would use to kick a soccer ball. Up to four weeks after familiarization, participants completed the first testing visit (visit two). Visit two consisted of anthropometrics followed by various assessments before and immediately after the completion of unilateral eccentric exercise designed to produce EIMD. Assessments included B-mode ultrasound imaging, self-reported soreness, palpation-based soreness, and neuromuscular assessment measures. The third, fourth, and fifth visits occurred 24-, 48-, and 72-hours post-EIMD, respectively, and included all assessments performed during visit two. Each visit took place at the same time of day (± 2 hours). The familiarization, 24-, 48-, and 72-hours post-EIMD visits lasted approximately one hour while visit two lasted approximately two hours. B-mode ultrasound was performed on the vastus lateralis (VL) and rectus femoris (RF) to obtain measures of muscle size and quality. Self-reported soreness was determined from a visual analog scale (VAS) of 0 (no pain) to 10 (extreme pain). For the palpation, participants were seated in the isokinetic dynamometer and the same investigator used four fingers to apply consistent pressure against the proximal, midpoint, and distal aspects of the quadriceps. Participants provided feedback for the palpation at each site on the aforementioned VAS scale.[15,24]
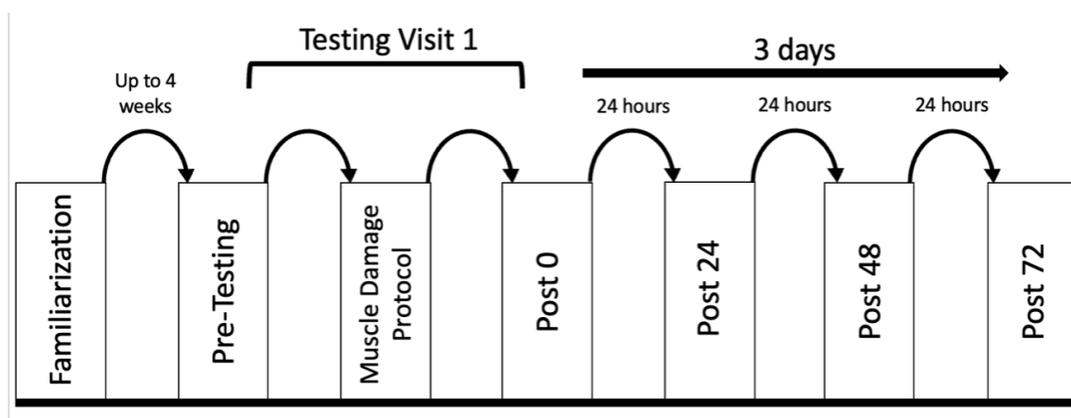

Figure 2. Timeline of the Study. Post 0: Immediately after the damage protocol. Post 24: 24 hours after the damage protocol. Post 48: 48 hours after the damage protocol. Post 72: 72 hours after the damage protocol.

Ultrasound Imaging.

B-mode ultrasound scans were taken on the VL and the RF during each visit to the laboratory except for the familiarization visit. Images were taken using an ultrasound imaging device (LOGIQ e R10.1; General Electric Company, Boston, MA, USA) and a multi-frequency linear-array probe (L4 – 12t - RS, 4.2–13 MHz, 47.1 mm field of view; General Electric Company, Milwaukee, WI, USA). Before the ultrasound assessment, participants laid supine for at least 10 mins to allow for fluid distribution. Participants laid supine on a padded table for the full duration of the ultrasound assessment. To assess measures of muscle size and quality (i.e., echo intensity [EI] and

cross-sectional area [CSA]), transverse ultrasound scans were taken at 50% of the muscle length. The muscle length was determined by the proximal and distal musculotendinous junctions measured using flexible tape and marked with a permanent marker. Sufficient acoustic coupling gel (Aquasonic 100 ultrasound transmission gel, Parker Laboratories, Inc., Fairfield, NJ, USA) was placed on the leg. Throughout the duration of the scans, minimal and consistent pressure was applied to the participant by the investigator. Ultrasound settings remained consistent throughout all assessments: gain of 52 dB, depth of 6 cm, and a frequency of 12 MHz. Echo intensity values were corrected for subcutaneous fat thickness using the equation by Young et al.[25] Image analysis was performed using the polygon and histogram tools on an open-source image analysis software (ImageJ, National Institutes of Health, Bethesda, MD). The polygon tool was used to assess CSA and in conjunction with the histogram function, EI was evaluated by computer-aided gray-scale analysis. The EI was determined as the mean pixel brightness value from 0-255 a.u. (0 = true black, 255 = pure white).

Isometric Strength Assessments.

Maximal voluntary contractions and maximal RT were used as metrics of neuromuscular function. All strength assessments were performed on a commercial, calibrated isokinetic dynamometer (HUMAC NORM, CSMi, Stoughton, MA, USA). Isometric muscle actions were used to assess the maximal strength of the knee extensors before and after the muscle damage protocol and throughout recovery. The lower leg was secured with a Velcro strap placed above the lateral malleolus. The axis of rotation on the dynamometer was aligned with the center of the participant's knee. Seatbelts were also securely fastened across the waist and chest of the participant (Figure 3). Participants performed three warm-up muscle actions, two at 50% and one at 75% of their perceived maximal strength. After the warm-up, the participants performed three maximal effort trials separated by two minutes of rest. For all of the warm-up and maximal trials, the knee joint angle was set at 1.05 rads below the horizontal plane of the lever arm and had a duration of 3-5 seconds. For the maximal trials, participants were told to kick out as hard as possible. They also received verbal encouragement throughout the duration of each maximal trial. The variable that was assessed from the three trials was peak torque. The highest peak torque from the trials was selected for analysis.

Figure 3. Isokinetic Dynamometer Chair Set Up. Picture shows the set up used for participants in the isokinetic dynamometer with a knee joint angle of 60°.

In addition to peak torque, RT was assessed through rapid isometric muscle actions in which the primary objective was for the participants to generate force as fast as possible. Participants were given a visual guide representing 80% of their peak torque. They were instructed to generate enough force to meet that target as fast as possible. They completed three rapid muscle actions trials separated by one minute of rest. RT and MVC were assessed from separate trials to allow for participants to achieve maximal RT and peak torque individually.[26] The average RT of the three trials was used for analysis.[26]

Isokinetic Strength Assessment.

In addition to isometric strength assessments, participants performed maximal isokinetic eccentric muscle action strength testing. Following the isometric strength assessments, participants remained seated in the chair and performed three ECC leg extensor exercises. Participants began with their knee joint angle at 1.83 rads of flexion. To start the task, participants were told to kick up until the knee joint angle was at 0.70 rads of flexion. Once the participant had kicked up to 0.70 rads of flexion, the dynamometer arm was programmed to move the knee joint to 1.83 rads of flexion (1.53 rads range of motion) at 1.57 rads/second. The participants were instructed to resist the downward motion of the dynamometer arm as hard as they could until the knee joint angle returned to 1.83 rads of flexion. Each repetition was separated by one minute of rest. This assessment was utilized to provide isokinetic eccentric peak torque. For analysis, the average peak torque of the three trials was used.

Signal Processing.

A custom-written software program (LabVIEW 23.3; National Instruments, Austin, TX, USA) was designed to process the aforementioned torque signals offline. Torque signals were calibrated and 0-meaned, followed by the application of a lowpass Butterworth filter with a 150 Hz cutoff frequency. Isometric peak torque was determined from the highest 500 ms epoch within the plateau region of the MVC. RT was quantified at 25 ms (RT_{25}), 50 ms (RT_{50}), 100 ms (RT_{100}), 200 ms (RT_{200}), and 250 ms (RT_{250}) from the rapid MVCs. Torque onset was manually determined by the same investigator by selecting the time point in which the torque began to deflect upward from baseline. Eccentric peak torque (ECC_{PT}) was determined from the highest 500 ms epoch from each repetition.

EIMD Protocol.

Participants completed 8 sets of 10 maximal-effort unilateral eccentric knee extensions on an isokinetic dynamometer using their dominant leg. Eccentric actions were performed at 1.57 rad/s, and the researcher assisted the return to the starting position during the faster concentric phase (6.98 rad/s). The range of motion spanned from 0.70 to 1.83 rad of knee flexion. One-minute rest intervals separated each set to minimize fatigue, and participants received consistent verbal encouragement throughout.

Statistical Analysis

All descriptive data are reported as the mean ± standard deviation (SD). A family-wise alpha level of $p \leq 0.05$ was used to determine statistical significance. *All data were visually inspected for normality using histograms and Q-Q plots. In addition, skewness and kurtosis were examined to assess possible deviations from normality.* All statistical analyses were performed using custom-written scripts in R (R version 4.2.1 (2022-06-23) -- "Funny-Looking Kid").

For all dependent variables, a 2 (age [older, younger]) x 5 (time [PRE, Post 0, Post 24, Post 48, Post 72]) mixed factorial ANOVA was used to compare age groups by time. The *F* statistic was used to compare variance. Effect size is reported using partial eta squared (η_p^2) with 0.01 indicating a small effect, 0.06 indicating a medium effect, and 0.14 indicating a large effect.[28] Tukey's post-hoc analyses were utilized in the event of a significant main effect of time or interaction effect.

Results

Neuromuscular Performance

The outcomes from the peak torque ANOVA did not indicate a significant age x time interaction effect ($F = 0.25$, $\eta_p^2 = 0.01$, $p = 0.91$) or a significant main effect for time ($F = 0.40$, $\eta_p^2 = 0.02$, $p = 0.81$). When collapsed across all time points, there was a significant main effect for age ($F = 33.29$, $\eta_p^2 = 0.33$, $p < 0.001$), with the younger (287.87 ± 65.30 Nm) females having a higher peak torque than the older (205.17 ± 46.06 Nm) females (Table 2).

For the change in RT_{25}, there was no significant age x time interaction ($F = 0.79$, $\eta_p^2 = 0.04$, $p = 0.54$). Additionally, there was no significant main effect for time ($F = 1.14$, $\eta_p^2 = 0.06$, $p = 0.34$) or age ($F = 0.18$, $\eta_p^2 = 0.003$, $p = 0.67$). Similarly, for RT_{50}, there was not an age x time interaction ($F = 0.34$, $\eta_p^2 = 0.02$, $p = 0.85$), nor significant main effects found for time ($F = 1.003$, $\eta_p^2 = 0.06$, $p = 0.41$) or age ($F = 1.28$, $\eta_p^2 = 0.02$, $p = 0.26$).

The ANOVA outcomes for the change in RT_{100} did not result in a significant age x time interaction effect ($F = 1.04$, $\eta_p^2 = 0.06$, $p = 0.39$) or a significant main effect for time ($F = 0.74$, $\eta_p^2 = 0.04$, $p = 0.57$). Conversely, there was a significant main effect for age ($F = 46.85$, $\eta_p^2 = 0.41$, $p < 0.001$) in which the younger (189.85 ± 37.35 Nm) females demonstrated higher RT_{100} than the older (122.76 ± 47.80 Nm) females (Figure 4).
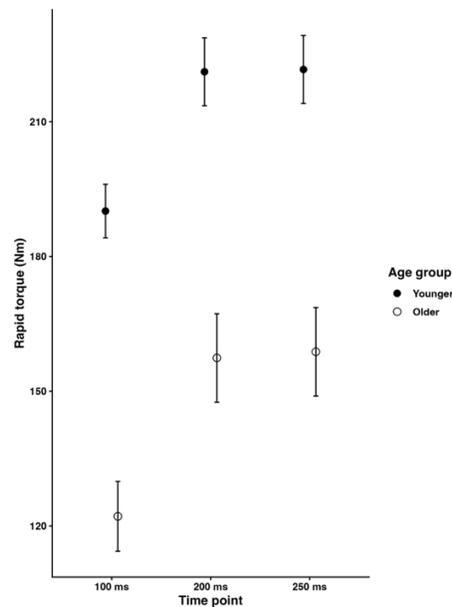

Figure 4. Rapid Torque at 100ms, 200ms, and 250ms. Estimated marginal means for rapid torque (Nm) at 100 ms (A), 200 ms (B), and 250 ms (C) for ten younger (20 ± 2 years, 22.49 ± 3.97 kg/m²) and six older females (68 ± 2 years, 24.45 ± 2.82 kg/m²). The data is shown for both groups when collapsed across all time points following a muscle damage protocol consisting of 8 sets of 10 repetitions of maximal eccentric muscle actions. * Indicates a significant main effect for age.

The results from the change in RT$_{200}$ ANOVA indicated a significant age x time interaction effect (F = 3.95, η_p^2 = 0.09, p < 0.001) (Figure 5). Upon performing the Tukey's- p-value adjustments, there were significant age x time pairwise comparisons between the older females at post 72 to the younger females at all time points (p = 0.005 - 0.039) with the older females performing worse than the younger females (97.22 – 118.29 Nm difference). There was no main effect for time found (F = 0.93, η_p^2 = 0.05, p = 0.45), but there was for age (F = 25.14, η_p^2 = 0.27, p < 0.001). The younger (220.88 ± 44.21 Nm) females exhibited higher RT than the older (158.56 ± 67.02 Nm) females at 200 ms (Figure 5).

The outcomes from the ANOVA for the change in RT$_{250}$ indicated a significant age x time interaction effect (F = 3.87, η_p^2 = 0.09, p < 0.001) (Figure 5). Upon performing the Tukey's- p-value adjustments, there were significant pairwise comparisons between the older females at post 72 to the younger females at all time points (p = 0.005 – 0.039) with the older females exhibiting lower RT than the younger females (97.62 – 118.31 Nm difference) . Furthermore, there was not a significant main effect for time (F = 0.96, η_p^2 = 0.05, p = 0.44). After collapsing across time points, there was a main effect for age (F = 24.37, η_p^2 = 0.26, p < 0.001), with the younger (221.40 ± 44.26 Nm) females producing greater RT$_{250}$ than the older (159.97 ± 67.08 Nm) females (Figure 5).
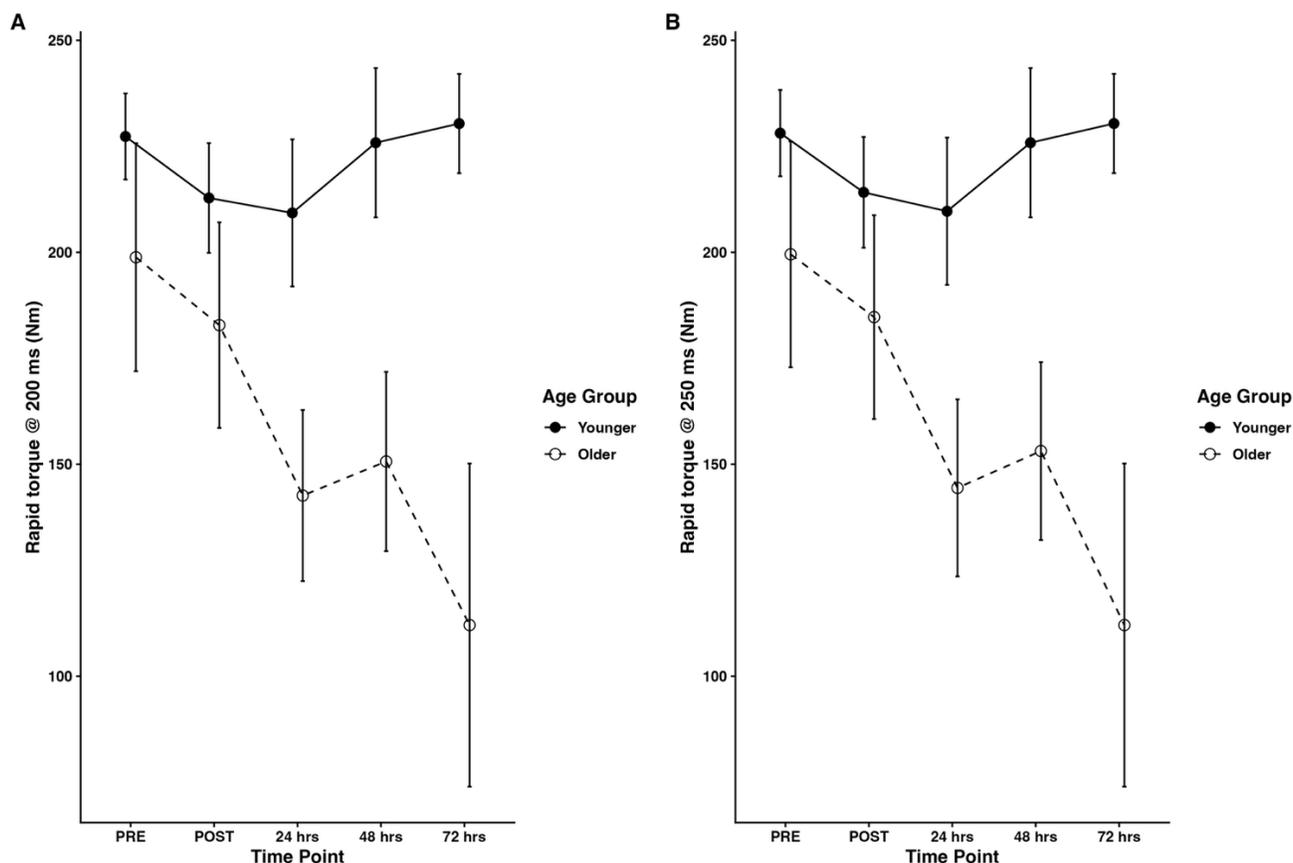

Figure 5. Rapid Torque at 200 ms and 250 ms. Box-plot representation of rapid torque produced at 200 ms (A) and 250 ms (B) across all time points for ten younger (20 ± 2 years, 22.49 ± 3.97 kg/m^2) and six older females (68 ± 2 years, 24.45 ± 2.82 kg/m^2). The data shown for five time points, immediately before (PRE) a muscle damage protocol consisting of 8 sets of 10 repetitions of maximal eccentric muscle actions, immediately after (POST), 24 hours after (24 hrs), 48 hours after (48 hrs), and 72 hours after (72 hrs) the muscle damage protocol. The data is represented by the group average and interquartile range. * Indicates a significant difference compared to the younger group at all time points

For the ECC_{PT}, the ANOVA did not show a significant age x time interaction effect ($F = 0.96$, $\eta_p^2 = 0.05$, $p = 0.43$) or a main effect for time ($F = 0.43$, $\eta_p^2 = 0.02$, $p = 0.79$). A main effect for age was found ($F = 23.56$, $\eta_p^2 = 0.26$, $p < 0.001$), with the younger (340.07 ± 80.44 Nm) females demonstrating greater ECC_{PT} than the older (247.97 ± 77.94 Nm) females.

Table 2. Peak Torque Averages. Group averages of peak torque for ten younger (20 ± 2 years, 22.49 ± 3.97 kg/m²) and six older females (68 ± 2 years, 24.45 ± 2.82 kg/m²). The data is shown for five time points, immediately before (Pre) a muscle damage protocol consisting of 8 sets of 10 repetitions of maximal eccentric muscle actions, immediately after (Post 0), 24 hours after (Post 24), 48 hours after (Post 48), and 72 hours after (Post 72) the muscle damage protocol. The data is represented by (Mean ± SD). * Indicates a significant main effect for age.

	Pre	Post 0	Post 24	Post 48	Post 72
Peak Torque (Nm)					
*Younger	293.84 ± 59.14	263.00 ± 64.65	290.49 ± 77.19	297.38 ± 70.80	295.61 ± 59.95
Older	220.38 ± 47.89	203.53 ± 45.79	200.14 ± 45.57	200.18 ± 44.72	201.11± 61.78

Muscle Size and Quality

The outcomes from the ANOVA did not indicate a significant age x time interaction effect for the change in VL CSA ($F = 0.03$, $\eta_p^2 = 0.001$, $p = 0.99$) or main effect for time ($F = 0.013$, $\eta_p^2 = 0.004$, $p = 0.06$). After collapsing across time points, there was, a main effect for age ($F = 24.93$, $\eta_p^2 = 0.268$, $p < 0.001$), with younger (16.99 ± 4.27 cm²) females having larger VL CSA than older (12.29 ± 2.85 cm²) females. For the change in RF CSA, the ANOVA did not indicate a significant age x time interaction effect ($F = 0.24$, $\eta_p^2 = 0.001$, $p = 0.99$) or a main effect for time ($F = 0.29$, $\eta_p^2 = 0.01$, $p = 0.88$). When collapsed across all time points, there was a main effect for age ($F = 33.28$, $\eta_p^2 = 0.33$, $p < 0.001$) with the younger (7.43 ± 2.19 cm²) females having larger RF CSA than the older (4.93 ± 1.07 cm²) females (Table 3).

The ANOVA outcomes for VL EI did not show a significant age x time interaction effect ($F = 0.10$, $\eta_p^2 = 0.01$, $p = 0.98$). Additionally, there was no main effect for time ($F = 0.39$, $\eta_p^2 = 0.01$, $p = 0.99$) or age ($F = 0.10$, $\eta_p^2 = 0.01$, $p = 0.53$). Similarly, RF EI did not indicate a significant age x time effect ($F = 0.11$, $\eta_p^2 = 0.007$, $p = 0.98$) or a significant main effect for time ($F = 0.01$, $\eta_p^2 = 0.004$, $p = 0.99$). However, a significant main effect for age was found ($F = 4.94$, $\eta_p^2 = 0.07$, $p = 0.03$). The younger females had a lower (127.88 ± 23.83 a.u.) echo intensity than the older females (140.51 ± 21.57 a.u.) (Table 3).

Table 3. Muscle Size and Quality Values. Group averages of muscle cross sectional area (CSA) and muscle echo intensity (EI) in the vastus lateralis (VL) and rectus femoris (RF) of ten younger (20 ± 2 years, 22.49 ± 3.97 kg/m²) and six older females (68 ± 2 years, 24.45 ± 2.82 kg/m²). The data is shown for five time points, immediately before (Pre) a muscle damage protocol consisting of 8 sets of 10 repetitions of maximal eccentric muscle actions, immediately after (Post 0), 24 hours after (Post 24), 48 hours after (Post 48), and 72 hours after (Post 72) the muscle damage protocol. The data is presented as Mean ± SD. * Indicates a significant main effect for age.

	Pre	Post 0	Post 24	Post 48	Post 72
Muscle VL CSA (cm²)					
*Younger	16.69 ± 4.60	17.31 ± 4.64	17.05 ± 4.49	17.15 ± 4.51	16.703 ± 3.90
Older	11.81 ± 3.09	12.55 ± 3.09	12.52 ± 3.00	12.47 ± 2.47	12.32 ± 3.24
Muscle RF CSA (cm²)					
*Younger	6.99 ± 2.25	7.43 ± 2.60	7.59 ± 2.07	7.78 ± 1.97	7.37 ± 2.26
Older	4.45 ± 1.06	4.92 ± 1.29	5.03 ± 1.02	4.91 ± 1.09	4.87 ± 1.17
Muscle VL EI (a.u.)					
Younger	128.12 ± 22.75	130.36 ± 17.57	131.00 ± 23.49	132.09 ± 24.02	127.22 ± 21.94
Older	131.90 ± 23.86	137.85 ± 25.07	130.14 ± 25.38	132.21 ± 22.90	134.05 ± 25.16

Muscle RF EI (a.u.)

*Younger	127.37 ± 24.60	129.16 ± 21.73	126.93 ± 25.33	131.02 ± 27.48	124.48 ± 24.40
Older	138.59 ± 23.11	143.97 ± 23.22	138.600 ± 22.45	138.317 ± 21.13	143.43 ± 26.06

Soreness

For the qualitative metric of self-reported soreness, the outcomes of the ANOVA did not indicate a significant age x time effect ($F = 1.95$, $\eta_p^2 = 0.09$, $p = 0.19$). There was a significant main effect for time ($F = 2.73$, $\eta_p^2 = 0.14$, $p = 0.04$), where soreness was elevated immediately post (1.44 ± 2.22 cm) compared to pre-testing (0.14 ± 0.28 cm) and remained elevated at post 24 (1.47 ± 2.17 cm) and then began to decrease at post 48 (1.29 ± 1.58 cm) and at post 72 (0.46 ± 0.59 cm). However, Tukey's-adjusted pairwise comparisons did not reveal statistically significant differences between time points. Additionally, a main effect for age ($F = 11.85$, $\eta_p^2 = 0.15$, $p < 0.001$) was present with the younger (1.41 ± 1.94 cm) females reporting greater soreness than the older females (0.23 ± 0.39 cm) (Figure 6).

For the change in proximal palpated soreness, the ANOVA did not indicate a significant age x time interaction effect ($F = 0.70$, $\eta_p^2 = 0.04$, $p = 0.60$) or a significant main effect for time ($F = 1.28$, $\eta_p^2 = 0.07$, $p = 0.29$). There was a significant main effect for age ($F = 5.32$, $\eta_p^2 = 0.07$, $p = 0.02$), with the younger (0.38 ± 0.68 cm) females reporting greater proximal soreness than the older (0.09 ± 0.13 cm) females (Figure 6).

The ANOVA result for the change in midpoint palpated soreness did not indicate a significant age x time interaction effect ($F = 1.05$, $\eta_p^2 = 0.06$, $p = 0.39$) or a main effect for time ($F = 2.15$, $\eta_p^2 = 0.11$, $p = 0.08$). When collapsed across all time points, there was a significant main effect for age ($F = 8.51$, $\eta_p^2 = 0.11$, $p = 0.004$) where the younger (0.53 ± 0.82 cm) females reported greater midpoint palpated soreness than the older (0.10 ± 0.14 cm) females (Figure 6).

The outcomes from the ANOVA for the change in distal palpated soreness did not show a significant age x time interaction effect ($F = 1.20$, $\eta_p^2 = 0.07$, $p = 0.32$) or a main effect for time ($F = 2.39$, $\eta_p^2 = 0.12$, $p = 0.06$). However, there was a significant main effect for age ($F = 12.04$, $\eta_p^2 = 0.15$, $p < 0.001$) where the younger (0.77 ± 1.08 cm) females reported greater distal palpated soreness than the older (0.10 ± 0.19 cm) females (Figure 6).

Discussion

The natural process of aging is associated with many physiological (mal)adaptations which may negatively influence neuromuscular function and performance.[7,29] Additionally, unaccustomed eccentric muscle actions have also been associated with decrements in performance.[11,12,30] However, the effect of age on neuromuscular performance following eccentric muscle actions remains uncertain. Despite the available muscle damage research, this study appears to be the first to investigate the effect of age on lower body neuromuscular performance following EIMD in older and younger females. The primary aim of this study was to evaluate the effect of age on the recovery time course of neuromuscular performance following eccentric EIMD. A secondary aim of this study was to quantify the effect of age on muscle size and quality following EIMD. Lastly, a tertiary aim of this study was to determine the effect of age on subjective metrics of muscle damage (soreness and palpation) following EIMD. The main effects for age indicated greater neuromuscular performance in peak torque, late phase RT, and ECC$_{PT}$ by the younger females compared with the older females. The absence of main effects for time and interaction effects for PT, early phase RT, and ECC$_{PT}$, suggest that the older and younger females responded
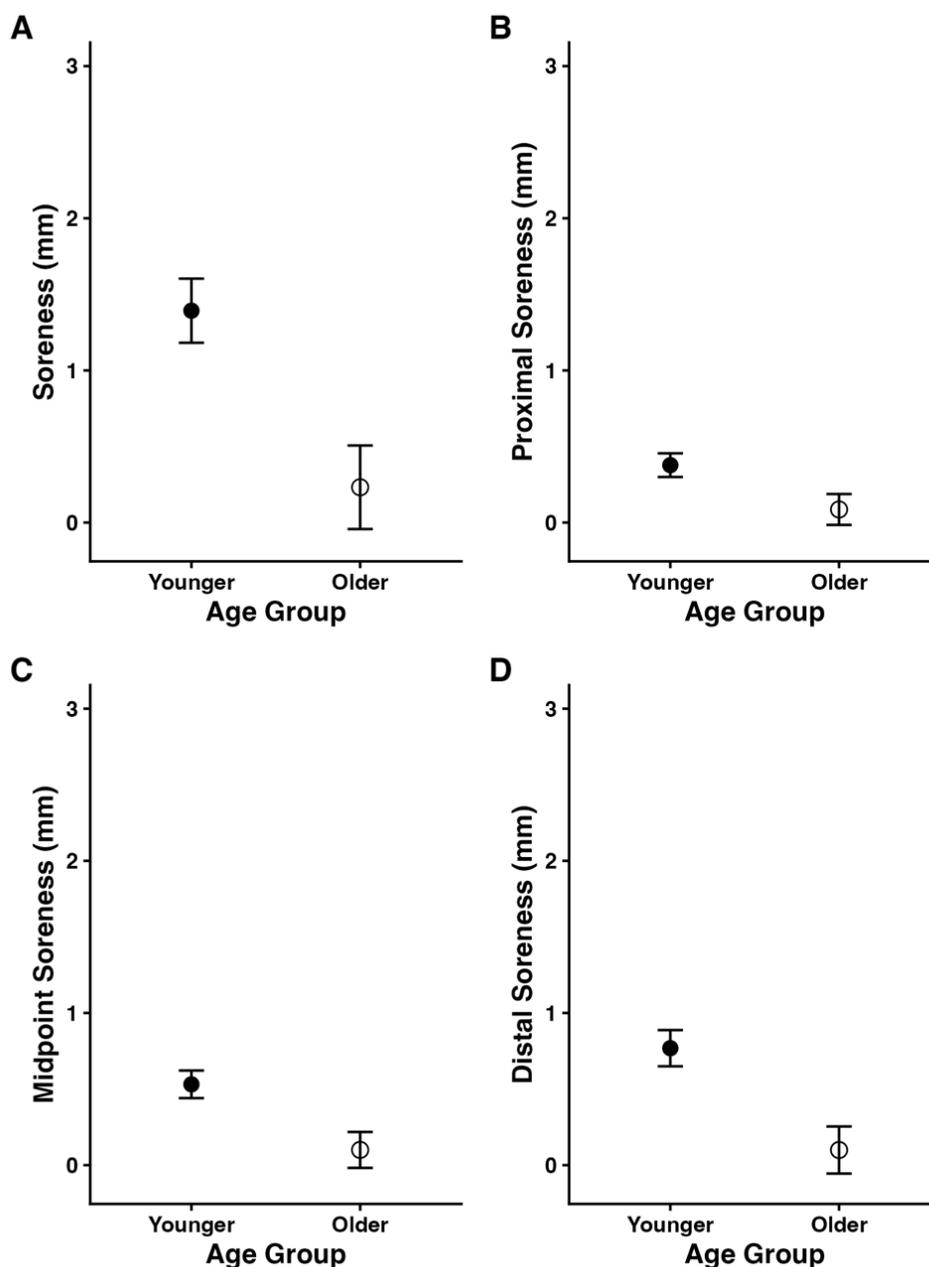
Figure 6. Soreness and Palpation. Estimated marginal means of soreness (A), proximal palpation (B), midpoint palpation (C), and distal palpation (D). The markers of soreness and palpation were reported on a 10 cm VAS scale. The groups consisted of ten younger (20 ± 2 years, 22.49 ± 3.97 kg/m²) and six older females (68 ± 2 years, 24.45 ± 2.82 kg/m²). The data is shown for the younger and older females when collapsed across all time points following a muscle damage protocol consisting of 8 sets of 10 repetitions of maximal eccentric muscle actions. * Indicates a significant main effect for age.

to and recovered from the muscle damage protocol similarly. However, when assessing late phase RT, there was a significant group x time interaction effect suggesting a slower recovery in the older females. Additionally, in a secondary analysis, a one-way ANOVA for the older females resulted in a significant effect of time at post-72 for RT_{200} ($p = 0.036$) and RT_{250} ($p = 0.034$). The main effects for age regarding muscle size (CSA) indicated greater VL and RF CSA in the younger females compared to the older females. However younger females only demonstrated

better muscle quality in the RF and not the VL. Finally, older females reported lower values for all subjective (self-reported and palpated soreness) metrics of muscle damage.

Maximal Strength

The outcomes from the current study suggest that peak torque was not reduced following repeated, eccentric muscle actions. Additionally, peak torque in the days following EIMD did not differ by age; however, the younger females demonstrated greater peak torque than the older females. Previous studies[16,18,31] have assessed maximal strength following EIMD in older populations. Contrary to the current study, previous findings have shown significant declines in maximal strength immediately following a muscle damage protocol, but the time course of recovery differed amongst studies.[16,18,31] Lavender & Nosaka[16] reported a quicker recovery in older males (returned to 87% of pre-muscle damage strength) than younger males (returned to 64% of pre-muscle damage strength) at day 10 following a dumbbell protocol of 30 elbow flexor muscle actions lasting five seconds each at 40% of the participants maximal isometric strength. A main difference between the earlier study by Lavender & Nosaka[16] and the present study is the sex of the sample. In an additional previous study, Dedrick & Clarkson[18] reported a difference in the recovery time course based on age, with younger females returning to pre-muscle damage strength sooner than older females. The authors[18] implemented a weight tray pulley system protocol comprised of 24 eccentric elbow flexor muscle actions lasting three seconds at 115% of the participants' maximal strength. Sex may be an important consideration since there are sex differences in the prevalence of dynapenia in older adults. Benjumea et al.[32], reported that dynapenia was present in 84.6% of older females tested and only present in 71.2% of older males tested. The difference in the presence of dynapenia may contribute to older women generating less absolute force, and therefore less mechanical strain, during maximal effort muscle actions. By generating less force, the extent to which muscle damage occurs may be diminished compared to older males. For the younger adults, a possible difference in the findings between males and females could be the onset of fatigue. Young females have shown to be less susceptible to fatigue during slow velocity dynamic muscle actions as opposed to young males. The current study as well as previous literature[16,18] have been unable to distinguish muscle fatigue from muscle damage when assessing maximal strength immediately following a muscle damage protocol. Also, in contrast to the current study, previous studies[16,18] assessed the elbow flexors as opposed to the knee extensors. Chen et al.[33] reported greater overall susceptibility to muscle damage in the elbow flexors than the knee extensors in all measures of muscle damage, including peak torque, following maximal eccentric muscle actions. The results from Chen et al.[33] could explain the differences in results seen in studies testing the upper body versus the lower body. In support of the present findings, Heckel et al. [31] reported a similar time course for recovery between the older and younger males following muscle damage. Heckel et al.[31] implemented a protocol on an isokinetic dynamometer consisting of 60 maximal eccentric muscle actions at 1.05 rads/second, a slower velocity than the present study. Overall, the findings from previous literature suggests that age may affect the response to and recovery from eccentric muscle actions. However, when considering the current findings in conjunction with previous studies, the magnitude of damage and the time course of recovery in different age groups remains uncertain. Further investigation on the impact of sex, muscle groups, and protocol intensity on muscle damage and recovery is still needed.

Rapid Strength

The findings from this study indicated greater rapid torque production and a faster recovery

for late phase RT (i.e., 200 and 250 ms) in the younger females than the older females. Rapid torque has been suggested as a more sensitive metric of muscle damage compared to isometric peak torque due to the difference in contribution of neural and muscular factors.[26] Previous work[12,34] has assessed early (e.g., 0-100 ms) and late phase RT (e.g., 100-200 ms) as performance metrics following muscle damage protocols. Peñailillo et al.[12] reported a 30-32% decrease in late phase RT from 100-200 ms following a 30-min eccentric cycling bout in young healthy men. The present results are supported by Peñailillo et al.[12] which also reported significant findings in late phase RT but not in early phase RT. However, unlike Peñailillo et al.[12], the present findings did not indicate significant declines in late phase RT immediately following EIMD. Early and late phase RT are impacted by different physiological factors which may explain the presence of significant late phase RT findings but not significant early phase RT. Early phase RT is more dependent on neural factors, such as motor unit firing rate, rather than MVC peak torque.[26] In contrast, late phase RT is impacted by muscular factors such as muscle size and architecture similarly to MVC peak torque.[26] The cycling protocol used by Peñailillo et al.[12] consisted of a greater exercise volume than the present study which may explain the significant decline in RT immediately following the muscle damage protocol in Peñailillo et al. but not the current study. The performance discrepancies in RT seen between age groups in this study is supported by Libardi et al.[34] who reported older adults having a peak knee extensor RTD ~50% lower than younger adults. Possible contributing factors to lower peak RTD in older adults include adverse changes in neural (i.e., decreased motor unit recruitment and firing rate) and muscular (i.e., type II muscle fiber denervation, muscle quality, and atrophy) mechanisms throughout aging.[7,26,34]

Muscle Size and Quality

The results from our study did not indicate any significant changes in muscle size or quality in either group at any time point following the muscle damage protocol. However, the older females had smaller overall muscle size and poorer muscle quality in the RF than the younger females. While ultrasound imaging metrics such as EI and muscle thickness have been previously utilized as a marker of muscle damage[19,35], the use of ultrasound assessed muscle CSA has not been previously investigated. Nosaka & Clarkson[19] reported significant increases in ultrasound assessed muscle size (muscle thickness) ranging from 0.6 cm to 2.2 cm young males following 24 maximal eccentric elbow flexor muscle actions. The difference in findings may be due to the different muscle groups assessed as well as the sex of the population. Consistent with previous literature, the present study found significant differences in muscle size and quality between the older and younger females[29,36]. Häkkinen & Häkkinen[36], assessed quadriceps femoris muscle CSA in younger, middle-aged, and older females. The older females in the study[36] exhibited significantly smaller CSA than the younger females. The difference in muscle CSA in older adults compared to younger adults is often attributed to sarcopenia, or the age-related loss in muscle mass, preferentially type II muscle fibers[4]. Similarly to dynapenia, sarcopenia is more prevalent in females and occurs most frequently when they are between 60-70 years old, but may begin as early as the 30's.[37]

In addition to the significant increase in muscle size, Nosaka & Clarkson[30] reported an increase in EI following EIMD. Similarly, Gonzalez-Izal et al.[35] found a significant increase in EI 48 hours after an eccentric protocol consisting of 4 sets of 20 repetitions of maximal eccentric knee extensor muscle actions in healthy young males and females. However, it may be important to note that the aforementioned studies [19,35] solely consisted of young adults, most of which were males. Additionally, the studies[19,35] did not adjust the EI values for subcutaneous fat thickness. Higher subcutaneous fat thicknesses result in a lower EI value due to the attenuation of the ultrasound

waves.[38] Therefore, the influence of subcutaneous fat may have impacted the reported EI values. In support of our findings, Fukumoto et al.[39] reported a significantly higher quadriceps femoris EI in older females compared to younger females. EI is strongly related to the amount of fibrous and non-contractile tissue within a muscle due to the high echogenicity of fat tissue and low echogenicity of fibrous tissue.[38] Throughout the lifespan, there is a natural loss in muscle mass and an infiltration of fat tissue. Over a five-year time period, Delmonico et al.[29] reported a 29% increase in intramuscular fat and a ~3% decrease in muscle size in females. The combination of an increase in intramuscular fat and the loss of fibrous tissue reported in older adults may contribute to the age-related differences in EI seen in the current study.

Perceived Soreness

In the present study, the younger adults reported higher measures of self-reported soreness than the older adults. Additionally, soreness was increased following the muscle damage protocol and began to decrease at 48 hours post protocol. Secondary analysis of a one-way ANOVA in the younger females resulted in significant differences in soreness in the younger females immediately post ($p = 0.012$), at post 24 ($p = 0.016$), and post 48 ($p = 0.03$) compared to pre-EMID values. Dedrick & Clarkson [18] reported a similar time course for the recovery of soreness, in which soreness remained elevated at 48 hours following the muscle damage protocol. However, in contrast to the present study, when comparing the magnitude of soreness reported between age groups, Dedrick & Clarkson [18] found no differences. The outcomes from the palpation-based assessments indicated a difference in soreness between age groups at all three independent palpation locations: proximal, midpoint, and distal. The current findings are supported by Lavender & Nosaka[16] who reported greater soreness when palpated in the younger males than the older males. In an additional study, the authors[24] tested three different eccentric elbow flexor protocols consisting of 12, 24, or 60 repetitions in which they did not find any correlations between reported soreness and other indirect measures of muscle damage such as peak torque or arm circumference. The differences seen in self-reported soreness between groups is often attributed to the individuality of each participant's perception of pain.[24] An important consideration when assessing palpation-based soreness is the possible difference between the amount of pressure applied between participants and time points despite an effort to control for discrepancies in the applied pressure. The findings of the present study, in conjunction with existing literature, suggest that soreness may depend on the individual's own perception of pain and, therefore, may not be the best indicator of the presence of muscle damage, despite its popularity.

A limitation of the study was the lack of control regarding diet and fluid consumption prior to the muscle damage protocol and throughout the recovery period. Recent work has suggested controversy on the influence of hydration status and total body water on EI values.[40] In addition, the muscle damage protocol that was used may not have been at a slow enough velocity or high enough volume to induce statistically significant differences across time points or between groups. Heckel et al.[31] utilized a slower velocity of 1.05 rads/sec as opposed to 1.57 rads/sec which resulted in significant differences between older and younger adults as well as significant differences across time points in various metrics of muscle damage. When assessing soreness, despite the attempt to control for differences in pressure by using the same investigator for all participants and time points, there still may be discrepancies. Finally, the primary limitation of this study was the number of participants who were tested. There were a total of 14 participants who completed this study, which is slightly below the sample size of previous muscle damage studies.[18,20,23] Despite our attempts to conservatively estimate the effect size used in the power analysis, this effect size may have been inflated. Also, the group sizes were uneven with nine

younger females and five older females. The smaller sample size in the older female group may have reduced our statistical power to detect significant changes in muscle strength recovery following the muscle damage protocol. Further research with larger sample sizes is needed to verify the presence (or absence) of EIMD-induced strength changes in both young and older adults.

The present study was the first to investigate age-related differences in lower body neuromuscular performance following EIMD in females. The results of this study add to the current literature supporting differences in neuromuscular function and performance between younger and older females. The difference between age groups is seen through the indication of an age effect in peak torque, late phase RT, ECC_{PT}, VL and RF CSA, RF EI, and soreness metrics. However, there were no differences seen amongst muscle quality and size, or peak torque and RT metrics in the pattern of recovery between age groups. Additionally, there were limited significant changes across all assessments seen following the muscle damage protocol in both groups. The main significant findings were changes in late phase RT and soreness metrics. Further investigations are needed to fully assess the effects of age on lower body performance following muscle damage by utilizing other modalities of muscle damage and different isokinetic dynamometer protocols.

When assessing the effect of age on the neuromuscular response to muscle damage, older and younger females responded similarly to EIMD. However, there were significant differences regarding neuromuscular performance and function between the age groups. Practitioners should remain aware of the decreased functional abilities of female clients as they age and prescribe exercises appropriately. Additionally, with the limited research investigating the effect of age on the response to EIMD in females, it may be beneficial for practitioners to use caution when prescribing unaccustomed eccentric exercise to older females, as this may increase their risk of falls and fall-related injuries.

Acknowledgements

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